Exhibit **L-27**
Project N .——— Appl. No. 09/558,421
B ok N .———
107

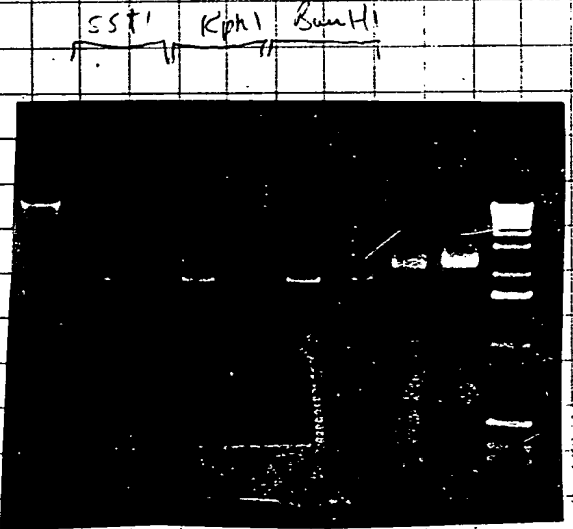ag N .— cut on at NdeI to see if all of luc is present even though
AatII is missing
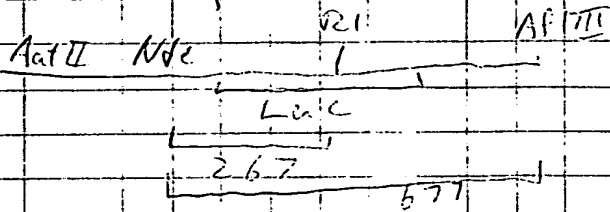
AatII Nde ──────┬──── AflIII   NdeII is OK in buffer 4 (NEB) (P169)
              V21   expect 677bp NdeI/AflIII band
        └── Luc ──┘
        └─── 267 ───┘
             └──── 577 ────┘

d cut with SSTI, KpnI or BamHI To see if missing
RI site is only a point mutation (are OK in NEB buffer 4)
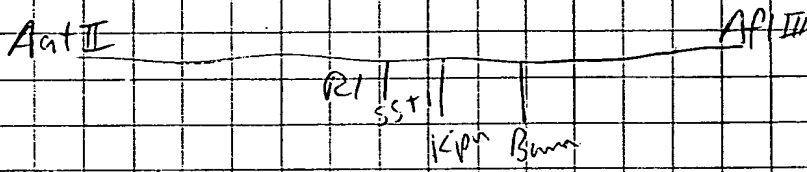
AatII ─────────────────────── AflIII   (for colony #20
         RI ┬ SSTI ┬   (tubes 2, 4, 6 below)
           SSTI   KpnI BamHI   see if SSTI, KpnI or Bam
                                   can cut in MCS

tube # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

SSTI KpnI BamHI

row	1	2	3	4	5	6	7	8		
A #1 (blue colony)		5	5	5	5				✓	
(missing R1)			5	5	5				✓	
missing AatII							5		✓	
t II	0.1 ──────────→ - - -								✓	
R1 III	0.3 ───────────────→								✓	
I	1	1								
n			1	1						
m HI				1	1					
e						1	1			
2 0	11.6 ──────────────→								✓	
B buffer 4	2 ──────────────→								✓	
	20µl									

37°C   2 hr
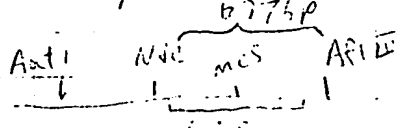

Result

G A A T T C G A G C T C   SSTI cuts but RI did not (P106)
   R1 ──── SSTI   is mutation is no more than 1 bp downstream
                            from the R1 site and may even be a
point mutation

NdeI may give the 677bp fragment expected   Aat I Nde mcs AflIII
but multiple bands are seen   └─ 677bp ─┘
                                                    Luc
                                              To Pag No.——

ssed & Understood by me,	Date	Invented by	Date
eer uara Bolao	2/16/95	[signature]	1-13-95
		R cord d by	